CHEMICAL ECOLOGY OF HONEYBEES IN ASIA: COMPARISON OF WORKER PHEROMONE GLAND COMPONENTS.

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Worker pheromone gland chemistry of several honeybee species in Asia, *Apis cerana japonica* (*Acj*), *A. c. cerana* (*Acc*), *A. c. indica* (*Aci*), *A. c. himalaya* (*Ach*), *A. dorsata* (*Ad*), *A. laboriosa* (*Al*) and *A. andreniformis* (*Aa*) was studied using GC/MS.

In the Nasonov gland extracts of *Acj*, *Acc*, *Aci*, and *Ach*, none of the compounds known as Nasonov pheromone in *A. mellifera* (*Am*) was detected. Instead, linalool oxide was identified as common component in *Acj* and *Acc*.

3-Hydroxyoctanoic acid was identified as a major mandibular component in foragers of *Acj*, *Aci* and *Acc*. Further studies using *Acj* revealed that the mandibular glands components change according to the division of labor (aging).

The sting gland components did not differ significantly between the species. Isopentyl acetate, the alarm pheromone in *Am*, was identified in the sting gland among all the species tested.

These results suggest that honeybees in Asia utilize different sets of chemicals as their Nasonov and mandibular glands pheromone from those of *Am*. On the other hand, alarm pheromone in the sting gland was suggested to be common language among the species.

SMALL-ANGLE X-RAY SCATTERING/DIFFRACTION OF PROTEINS LABELED WITH METAL CLUSTERS: APPLICATION TO MOTOR PROTEIN SYSTEMS.

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Small-angle X-ray scattering/diffraction technique is useful in obtaining structural information of proteins or protein systems functioning under near-physiological conditions. A shortcoming of this technique is its limited spatial resolution. One of the ways to overcome this shortcoming is to label the specific sites of the target protein with metal clusters, which scatter X-ray strongly and thus enhances the signals from the labeled sites. This improvement is expected to provide the capability to detect local movements of domains within a protein, to measure the distance between the two labeled sites within a protein or a protein complex, and so on. Preliminary studies using the actomyosin system is now underway. Gold clusters are introduced to myosin molecules at two sites. One is the highly reactive cysteine residue (Cys707), to which the cluster is linked covalently. The other is the ATP binding site. We synthesized an ATP with a covalently bound gold cluster at the ribose moiety. This ATP analog can be hydrolyzed by myosin, although at a lower rate. The optimum condition for recording X-ray scattering from labeled myosin or actomyosin complex is being sought using the high-intensity synchrotron radiation source at SPring-8.

CO-EVOLUTION OF THE PLANT AND THE INSECT :
THE ORIENTAL ORCHID "Kinn-ryou henn" (*Cymbidium floribundum* Lindl.) CONTROLS THE SOCIAL BEHAVIOR OF THE JAPANESE HONEYBEE, AND RECOGNITION OF THE JAPANESE AND EUROPEAN HONEYBEE.

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The Japanese honeybee (*Apis cerana japonica* Rad. : *Acj*) and the European honeybee (*Apis mellifera* L.: *Am*) share the same habitat in Japan. Very little is known about the physiology and the chemical ecology of the Japanese honeybee.

The red and white flower varieties of the oriental orchid (*Cymbidium floribundum* Lindl.: *Cf*) attract workers, drones, queens as well as the entire swarming colonies of *Acj*, but not of *Am*. This is due to the fact that the flower scent mimics the Nasonov and mandibular glands pheromone of *Acj*. Both Nasonov gland and mandibular glands extracts induced aggregation behavior in both species, but the GC profiles of the extract from *Am* and *Acj* were significantly different.

We report here for the first time (1) the identification of more than 16 semiochemical compounds ((S)-linalool, 4 types of linalool oxide, fatty acids) of *Cf* and Nasonov gland and of *Acj*, these compounds elicited antennal responses in GC-EAD experiments and aggregation behaviors in bioassays, (2) the identification of *Cf* original compounds (alkanals, gamma-lactones, alkanols) attract only *Acj*, (3) 2-heptanone and 3-hydroxyoctanoic acid were found in both *Cf* scent and *Acj* mandibular glands. (4) Differences between *Acj* and *Am* might be due to differences in their semiochemical components as well as their sensitivity to and recognition of these semiochemicals. (5) The amount of the odor components in *Cf* was sufficiently enough to attract *Acj*. (6) Attraction of drones to *Cf* flowers was due to the response to the Nasonov pheromone components in the *Cf* scent. (7) Queen pheromone of *Acj* was not detected in *Cf* scent.

It is interesting that a plant like *Cf* can affect the social behavior of *Acj*. The experiments using the other Asian honeybees such as *Apis cerana cerana* are underway.

HEMOLYMPH CYSTATIN (CYSTEINE PROTEASE INHIBITOR) RESPONSIBLE FOR *BOMBYX* CYSTEINE PROTEINASE (BCP) I I

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We have reported that three kinds of inhibitors capable of inhibiting the activity of BCP are present in the hemolymph. This time, we have purified these inhibitors; cystatins, to homogeneous forms. The purification was performed through four steps of conventional column chromatography and HPLC. Two of the three cystatins showed different N-terminal amino acid sequences, suggesting that there are at least two kinds of cystatins. On the other hand, antiserum against one of the purified cystatins reacted with three kinds of cystatins, suggesting that the three cystatins share common antigenic properties. On the base of amino acid sequences obtained, PCR cloning of the cDNA encoding cystatins were attempted. Several cDNA clones were selected positively. Further cloning and sequence analysis are in progress.

MAP2 and Tau differs in their microtubule association mechanism

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MAP2, Tau, and MAP4 share a homologous microtubule binding domain[1)2)3)]. MAP2 is reported to compete with MAP4 for microtubule surface[4)]. We have reported elsewhere the competition between PA_4T fragment (microtubule binding fragment of MAP4) and MAP2[5)]. Here, we further analysed the mechanism of the two competitions.

Microtubules were reconstituted from purified tubulin, varing concentrations of the PA_4T fragment, and a constant amount of MAP2 or Tau. MAP2(0.5 μ M) was dose-dependently released from the microtubule fraction by the addition of the PA_4T fragment in the added fragment concentration range of 0–5 μ M, while half amount of MAP2 still remained on microtubules in the presence of 20 μ M PA_4T fragment. On the other hand, 9 μ M Tau was completely released from microtubules by the addition of 6 μ M PA_4T fragment. The results suggested that MAP2 and Tau have different microtubules associating mechanisms. 1)H. Aizawa, *et al* (1990) J. Biol. Chem. 265,13849–13855 2)G. Lee, *et al* (1988) Science 239,285–288 3)S. Lewis *et al* (1988) Science 242,936–939 4)H. Aizawa, *et al* (1989) J. Biol. Chem.264, 5885–5890 5)71st Annual Meeting of the Japanese Biochemical Society.

ANALYSIS OF A cDNA ENCODING A CALCIUM BINDING PROTEIN IN THE DECAPOD CRUSTACEAN *Penaeus japonicus*.

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Many marine invertebrates including crustaceans deposit calcium carbonate crystals in hard tissues. In decapod crustaceans, this process of calcification has been shown to take place at postmolt stages. We have identified a mRNA (named *DD4*) which is predominantly expressed at postmolt stages during the molt cycle in the tail fan of the kuruma prawn *Penaeus japonicus*. Results of sequence analysis of a *DD4* cDNA clone have shown that it encodes a proline-rich acidic protein which exhibits sequence similarity to a *Drosophila* calcium binding protein calphotin which is specifically expressed in photoreceptor cells. A partial DD4 protein synthesized in *E. coli* bound $^{45}Ca^{2+}$. These results suggest that the DD4 protein may be involved in concentration of Ca^{2+} at the calcifying site of the exoskeleton. We will also present results of preliminary sequence analysis of *DD5* which is also predominantly expressed at postmolt stages in the tail fan.